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Dated 15 March 2000

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The Patent Office

Cardiff Road
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1. Your reference — GM/96148 GB

2. Patent application number *(The Patent Office will fill in this part)* — **9621902.7**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Allelix Biopharmaceuticals Inc.,
6850 Goreway Drive,
Mississauga,
Ontario,
Canada L4V 1V7.

Patents ADP number *(if you know it)* 07086861001

If the applicant is a corporate body, give the country/state of its incorporation — Canada

4. Title of the invention

NEUROTROPHIN ANTAGONIST COMPOSITIONS

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

BATCHELLOR, KIRK & CO.,

2 PEAR TREE COURT,
FARRINGDON ROAD,
LONDON EC1R ODS.

Patents ADP number *(if you know it)* 315001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	10
Claim(s)	1
Abstract	1
Drawing(s)	3

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Batchellor, Kirk & Co Date 21-10-96

BATCHELLOR, KIRK & CO.

12. Name and daytime telephone number of person to contact in the United Kingdom

MARCH, Gary Clifford
0171 253 1563

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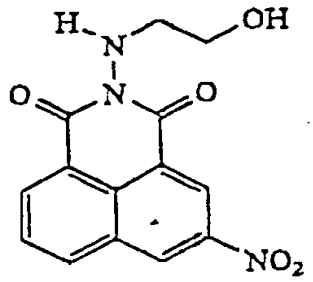
NEUROTROPHIN ANTAGONIST COMPOSITIONS

FIELD OF THE INVENTION

The present invention relates to neurotrophin antagonists. In particular, the present invention relates to compositions comprising an effective amount of a compound of Formula I, or pharmaceutically acceptable salts or *in vivo* hydrolyzable esters thereof, which inhibits or reduces undesirable neurotrophin activity, and a pharmaceutically acceptable carrier.



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BACKGROUND OF THE INVENTION

A family of structurally and functionally related neurotrophic factors exist which are collectively known as neurotrophins. The family of neurotrophins includes the nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), neurotrophin-5 (NT-5) and neurotrophin-6 (NT-6).

The neurotrophins exhibit similar structural conformations, including three surface ß-hairpin loops, a ß-strand, an internal reverse turn region, and N- and C- termini. With respect to sequence similarities, the neurotrophins share approximately 50% amino acid identity. The neurotrophins are also functionally similar in that they each exhibit low affinity binding to a receptor known as the "p75 nerve growth factor receptor" or $p75^{NGFR}$. Each neurotrophin also exhibits binding to a receptor of the tyrosine kinase (trk) family which is of higher affinity than the binding to the p75 receptor. This interaction is believed to be related to neuron survival, but is also involved with

neuronal differentiation including process formation. The trk receptor-neurotrophin interaction has been found to be more selective than neurotrophin interaction with the $p75^{NGFR}$ receptor. In particular, NGF binds only a trk receptor known as the TrkA receptor, while BDNF, NT-4 and NT-5 exhibit exclusive binding to a TrkB receptor. NT-3 is less selective and, although it binds primarily with a TrkC receptor, it also exhibits some binding to the TrkA and TrkB receptors (Ibanez et al., EMBO J. 1993, 12:2281).

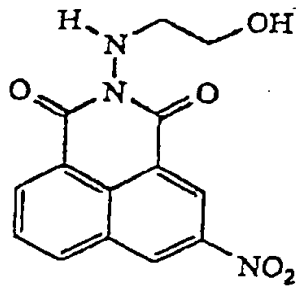
The neurotrophins function primarily to promote survival of certain classes of peripheral and central neurons both during development and following neuronal damage. NGF, in particular, is involved with the development of neurons in the peripheral nervous system and supports neuronal survival, as well as enhancing and maintaining the differentiated state of neurons. However, in some neurological disease states, the neurotrophins may also support inappropriate neurite outgrowth thereby facilitating the progression of a disease condition. For example, neurotrophins promote the undesirable sprouting of hippocampal "mossy fibres". Such inappropriate sprouting of mossy fibres is a common accompaniment of epilepsy in humans. It is also postulated that the pain experienced by patients suffering from some chronic pain syndromes may be associated with sprouting of sensory pain fibers responsive to NGF in particular into the spinal cord. In other pathological states, such as Alzheimer's disease, aberrant process growth, known as dystrophic neurite formation, is a strong correlate of disease severity.

Thus, although the neurotrophins are essential for the normal development and growth of neurons, they may be detrimental under certain circumstances. In such instances, ligands capable of inhibiting or reducing selected neurotrophin-mediated activities would be desirable therapeutically to treat neurodegenerative disease and repair nervous system injury.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide compositions capable of inhibiting, or at least reducing, undesirable neurotrophin-mediated activity.

In an aspect of the present invention, a composition is provided which comprises a carrier and an effective amount of a compound of Formula I:



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and pharmaceutically acceptable salts and *in vivo* hydrolyzable esters thereof.

In a further aspect of the present invention, there is provided a method for inhibiting a neurotrophin-mediated activity comprising the step of exposing neurons to a composition as described above.

A further aspect of the present invention provides a method for inhibiting neurotrophin-mediated activity in a mammal comprising the step of administering a composition as described above to said mammal.

These and other aspects of the present invention will be described in greater detail hereinbelow.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compositions comprising an effective amount of a compound of Formula I, or pharmaceutically acceptable salts or *in vivo* hydrolyzable esters thereof (hereinafter referred to as a compound of Formula I), which inhibits neurotrophin-mediated activity, and a pharmaceutically acceptable carrier.

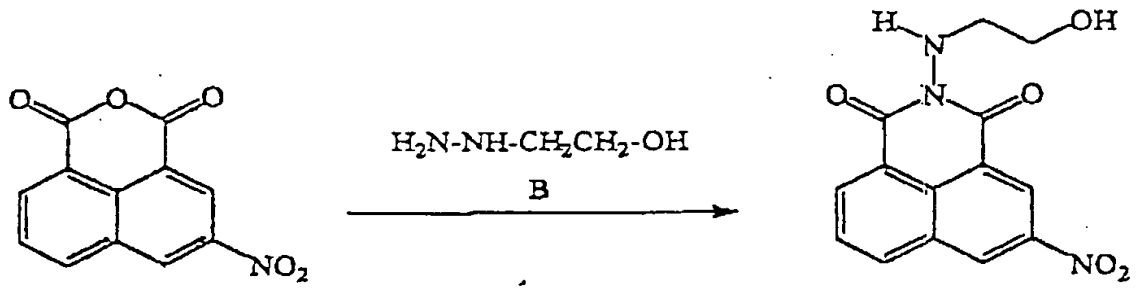
As used herein, *in vivo* hydrolyzable esters are those readily hydrolyzable esters, attached at the hydroxyl group of compounds of Formula I, which are known and used in the pharmaceutical industry and include α-acyloxyalkyl and esters of C_{3-20}-fatty acids.

As it is used herein, the term "neurotrophin" refers to neurotrophic factors that are structurally homologous to NGF, i.e. include three surface ß-hairpin loops, a ß-strand, an internal reverse turn region, and N- and C- termini, and which promote at least one of neuron survival and neuron differentiation, as determined using assays of conventional design such as the *in vitro* assay exemplified herein and described by Riopelle *et al.* in Can. J. of Phys. and Pharm., 1982, 60:707. Mammalian nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4 (NT-4), neurotrophin-5 (NT-5) and neurotrophin-6 (NT-6) are examples of neurotrophins.

"Neurotrophin-mediated activity" is a biological activity that is normally promoted, either directly or indirectly, in the presence of a neurotrophin. Neurotrophin-mediated activities include, for example, neurotrophin binding to the $p75^{NGFR}$ receptor or neurotrophin binding to one of the trk receptors, neuron survival, neuron differentiation including neuron process formation and neurite outgrowth, and biochemical changes such as enzyme induction. A biological activity that is mediated by a particular neurotrophin, e.g. NGF, is referred to herein by reference to that neurotrophin, e.g. NGF-mediated activity. To determine the ability of a compound to inhibit a neurotrophin-mediated activity, conventional *in vitro* and *in vivo* assays can be used. For example, a receptor binding assay, such as the assay described herein in Example 1, can be used to assess the extent to which a compound inhibits neurotrophin/receptor binding. Inhibition of neurite survival

and outgrowth can be determined using the *in vitro* assay described by Riopelle et al. in the Can. J. of Phys. and Pharm., 1982, 60:707, illustrated herein in Example 2.

The compound of the present invention is prepared by coupling naphthalic anhydride reagent A with hydrazino alcohol reagent B in an inert solvent at temperatures in the range of 80-120 °C. The preferred condition for this reaction is refluxing toluene. Both reagent A and reagent B are commercialy available or can be prepared using procedures known to one skilled in the art.



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Acid addition salts of the compound of Formula I are most suitably formed from pharmaceutically acceptable acids, and include for example those formed with inorganic acids e.g. hydrochloric, sulphuric or phosphoric acids and organic acids e.g. succinic, maleic, acetic or fumaric acid. Other non-pharmaceutically acceptable salts e.g. oxalates may be used for example in the isolation of the compound of Formula I for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt. Also included within the scope of the invention are solvates and hydrates of the invention.

The conversion of a given compound salt to a desired compound salt is achieved by applying standard techniques, in which an aqueous solution of the given salt is treated with a solution of base e.g. sodium carbonate or potassium hydroxide, to liberate the free base which is then extracted into an appropriate solvent, such as ether. The free base is then separated from the aqueous portion, dried, and treated with the requisite acid to give the desired salt.

In vivo hydrolyzable esters of the compound of Formula I can be formed by treating it with the acid chloride of the desired ester in the presence of a base in an inert solvent such as methylene chloride or chloroform. Suitable bases include triethylamine or pyridine.

The compositions of the present invention are useful to inhibit or reduce undesirable neurotrophin activity both *in vitro* and *in vivo*. Thus, in an aspect of the invention, a composition comprising an effective amount of a compound of Formula I and a suitable carrier is provided. By "suitable carrier" is meant a carrier which admixes with the compound of Formula I to yield a composition suitable for the application for which it is to be used. By "effective amount" is meant an amount of the compound sufficient to inhibit an undesired neurotrophin-mediated activity by about 50% as determined using assays of conventional design such as those described herein in the specific examples.

The present composition has use as a media supplement to prevent undesirable neurotrophin-mediated activity of neuron cells *in vitro*. For example, primary sensory neurons require NGF for survival in cell culture; however, NGF also influences neuron differentiation, notably process formation and outgrowth, which are undesirable for the use of primary sensory neurons in cell culture. Thus, to preserve neuron survival *in vitro* while inhibiting cell differentiation, NGF is added to the cell culture media along with the compound of Formula I. For addition to the cell culture, the compound is first combined with a carrier which will not adversely affect the growth of the cells in culture. Such carriers will include, for example, physiologically acceptable fluids such as water or any other fluid suitable for addition to the cell culture. Alternatively, the compound can be combined with media suitable for culturing neuronal cells prior to being added to the cell culture. To be effective to prevent neuron differentiation, the concentration of the compound in the cell culture will be in the range of from about 1-500 μM, and preferably from about 1-100 μM. The optimal concentration of compound for use in preventing neuron differentiation in cell culture will, of course, vary depending on the extent of inhibition desired as well as the type of neuronal cells involved.

Compositions for *in vivo* administration, e.g. for treating neurological conditions such as epilepsy or Alzheimer's disease, or for treating chronic pain, are also contemplated. Such compositions comprise a therapeutically effective amount of the compound of Formula I together with a pharmaceutically acceptable carrier. In this context, the term "pharmaceutically acceptable" means acceptable for use in the pharmaceutical and veterinary arts, i.e. non-toxic and not adversely affecting the activity of the compound. The term "therapeutically effective amount" means an amount of the compound sufficient to reduce undesirable neurotrophin-mediated activity, as determined using assays of conventional design, in an inflicted individual without causing adverse effects.

Pharmaceutically acceptable carriers useful to prepare compositions for *in vivo* administration include conventional carriers used in formulating drugs, such as diluents, excipients and the like. Reference may be made to "Remington's Pharmaceutical Sciences", 17th Ed., Mack Publishing Company, Easton, Penn., 1985, for guidance on drug formulations generally. As will be appreciated, the pharmaceutical carriers used to prepare compositions in accordance with the present invention will depend on the dosage form to be used to treat the inflicted individual.

According to one embodiment of the invention, a compound of Formula I is formulated for administration by injection intraventricularly, and is accordingly provided as an aqueous solution in sterile and pyrogen-free form and optionally buffered or made isotonic. Thus, the compound may be administered in distilled water or, more desirably, in saline or 5% dextrose solution. Water solubility of the compound of the invention may be enhanced, if desired, by incorporating into the composition a solubility enhancer, such as cetyltrimethylammonium bromide or chloride. Lyoprotectants, such as mannitol, sucrose or lactose and buffer systems, such as acetate, citrate and phosphate may also be included in the formulation, as may bulking agents such as serum albumin.

For use in treating individuals with a neurological condition, precise dosage sizes of a pharmaceutical composition appropriate for treatment are established in appropriately controlled trials, and will correspond to an amount of a compound of Formula I that reduces undesirable neurotrophin-mediated activity without causing intolerable side effects to the individual being

treated. It is anticipated that an effective treatment regimen for patients will involve the intraventricular administration of dosages which achieve a level of the compound in the spinal fluid of the individual being treated of about 1-500 μM. It will be appreciated, of course, that the dosage sizes required to attain this *in vivo* concentration will vary according to the route of administration, the frequency of administration, on the individual being treated and on the neurological condition being treated.

Specific embodiments of the present invention are described in more detail in the following examples which are not to be construed as limiting.

Example 1 - Binding of [^{125}I]NGF to PC12 cells in the presence and absence of BDNF

The ability of the compound of Formula I to antagonize NGF interaction with the p75 and trkA receptors was determined. PC12 cells were grown in RPMI with 10% heat inactivated donor horse serum and 5% fetal calf serum in the presence of additives. Cells were harvested by replacing the medium with calcium-magnesium-free phosphate buffer and incubated at 37 ^{0}C for 15 minutes. Cells were pelleted by centrifugation and suspended in HKR buffer (10 mM Hepes [pH 7.35] containing 125 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1 mg/mL BSA) at a concentration of $2x10^6$ cells/mL and kept at 4 ^{0}C. Each data point was set up in three tubes each containing 40 pM [^{125}I]NGF, competitor in the range from 0.03 μM to 100 μM, 400,000 cells (for a final concentration of 10^6 cells/mL) BDNF and NGF as required. The tubes were incubated for 2 hr at 4 ^{0}C. At the conclusion of the incubation 100 mL aliquots (providing duplicate data points for each tube, therefore, six data points for each sample) were transferred to 400 mL microcentrifuge tubes containing 100 mL of 10% glycerol in HKR. The tubes were centrifuged for 2 minutes at 10 000 g and the tip containing the cell pellete was cut off. Radioactivity bound to the cells was determined by measuring the [^{125}I]NGF associated with each pellet in a gamma counter. NGF was radioiodinated as described in Sutter *et al.* J. Biol. Chem. 1979, 254:5972. The Table below summarizes the IC_{50} values obtained from this experiment for the inhibition of binding of NGF to P75 and TrkA by the compound of Formula I.

Receptors	IC_{50} (μM)
P75 and Trk A	0.7
Trk A	2.8

Example 2 - Inhibition of Neurite Outgrowth

The ability of the compound of Formula I to inhibit neurite outgrowth was determined using the following biological assay.

Eight-day chick embryo dorsal root ganglia (DRG) were freed of meninges and removed aseptically. The DRG were kept at 4 °C at all times. Ganglia from six embryos (40-50 per embryo) were washed in Ca^{2+}- and Mg^{2+}- free Gey's balanced salt solution (Gibco) and exposed to 0.01% trypsin (Worthington) in the same solution for 10 min at 37 °C. A half-volume of phosphate-buffered Gey's balanced salt solution was added for a further 5 min at 37 °C and the reaction was then terminated with one-third volume of Ham's F12 medium (Gibco) containing 5% fetal calf serum (FCS, Gibco). The ganglia were then triturated using a 5 mL narrow-tip pipette to a single cell suspension. Following filtration through 37-mm nylon mesh (Small Parts Inc., Miami, FL) in a Millipore chamber to remove clumps, the cell suspension was washed through a 500-ml FCS undercut (700 x g for 5 min at 4 °C) and resuspended in 4 mL of Ham's F12 medium plus 5% FCS. The cell suspension was then preplated on a 100-mm Flacon culture dish and incubated for 45-60 min at 37 °C in a 5% CO_2 humidified atmosphere. Cells enriched in neurons were decanted for the bioassay, since non-neuronal cells of DRG preferentially stick to the culture substrate.

The inside wells of 96-well Falcon microculture plates were coated with polylysine (0.1 mg/mL) (Sigma) for 4 h at 37 °C (the outside wells were filled with distilled water to provide humidity) and, following a rinse with tissue culture media, 100 mL of neuron-rich cell suspension was added to each well at 10^5 cells/mL. Ninety (90) mL of NGF solution (prepared in tissue culture media) was then added to each well to a final concentration of 0.25 ng/mL NGF per well. Ten (10) mL of test compound solution, i.e. tissue culture media admixed with a compound of Formula I, was then added to test wells in duplicate to yield wells containing peptide concentrations

ranging from 0 μM - 100 μM. For control assays, 10 mL of Ham's F12 medium was added to duplicate NGF-containing wells. The plates were covered and incubated in the dark for 24-30 hrs. at 37 °C in a 5% CO_2 humidified atmosphere.

The bioassays were read using a Leitz Diavert microscope with phase optics. To afford adequate optics, the meniscus effect of each well was removed by filling the well with a balanced salt solution until a flat, air-filled interface was achieved at the top of the well. At least 100 neurons per well were counted, and the assay was scored as the ratio of cells bearing neurites greater than one cell diameter to total viable (phase bright) cells. These results are summarized in the Table below.

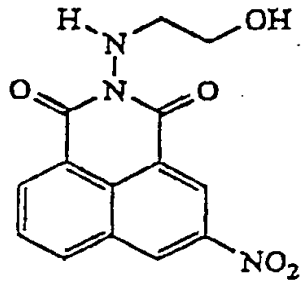
Group	Ratio	Std Dev	SEM
NGF Control	1.000	0.16	0.06
5 μM Cpd I	1.1	0.42	0.15
50 μM Cpd I	0.16	0.15	0.05
100 μM Cpd I	0.016	0.02	0.01

Example 3 - Preparation of N-[5-nitro-1H-benz[de]isoquinoline-1,3(2H)-dione]-2-aminoethanol

3-Nitro-1,8-naphthalic anhydride (1 eq) and 2-hydroxyethylhydrazine (1 eq) are dissolved in toluene and heated to reflux. The reaction is monitored by tlc and halted when all of the starting materials are consumed. The solvent is removed under reduced pressure and the product purified, if necessary, by recrystallization or silica gel chromatography.

WE CLAIM:

1. A pharmaceutical composition comprising a compound of Formula I, or pharmaceutically acceptable salts or *in vivo* hydrolyzable esters thereof, in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier.



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2. A pharmaceutical composition as defined in claim 1, which inhibits NGF-mediated activity.

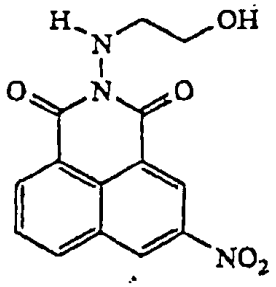
3. A method for inhibiting a neurotrophin-mediated activity comprising the step of exposing neuron cells to an effective amount of a composition as defined in claim 1.

4. A method for inhibiting a neurotrophin-mediated activity in a mammal comprising the step of administering to said mammal a therapeutically effective amount of a composition as defined in claim 1.

5. A method as defined in claim 4, wherein said composition is administered intraventricularly.

ABSTRACT

A pharmaceutical composition comprising a compound of Formula I or pharmaceutically acceptable salts or *in vivo* hydrolyzable esters thereof, in an amount effective to inhibit neurotrophin-mediated activity, and a suitable carrier is described.



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The compositions are useful to inhibit undesirable neurotrophin-mediated activity such as the neurite outgrowth that occurs in some neurodegenerative disease states.